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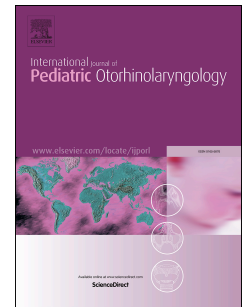
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**Experimental *Pseudomonas aeruginosa*  
mediated rhino sinusitis in mink**

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1

**2 Abstract.**

3 *Objectives:* The nasal and sinus cavities in children may serve as reservoirs for microorganisms  
4 that cause recurrent and chronic lung infections. This study evaluates whether the mink can be used  
5 as an animal model for studying *Pseudomonas aeruginosa* mediated rhino-sinusitis since there is no  
6 suitable traditional animal model for this disease.

7 *Methods:* Nasal tissue samples from infected and control mink were fixed in formalin,  
8 demineralized, and embedded in paraffin. A histological examination of sections from the infected  
9 animals revealed disintegration of the respiratory epithelium lining the nasal turbinates and swelling  
10 and edema of the submucosa. The expression of mucins and sialylated glycans was examined using  
11 immunohistochemistry.

12 *Results:* MUC1, MUC2 and MUC5AC were upregulated in the inoculated animals as a much  
13 stronger staining was present in the respiratory epithelium in the infected animals compared to the  
14 controls and MUC5B appeared in large macrophage-like cells in the submucosa. The goblet cells in  
15 the nasal epithelium from the infected mink showed high affinity to the *Maackia amurensis* lectin  
16 and anti-asialo GM1 indicating a high concentration of  $\alpha$ 2-3 sialic acid respectively  $\beta$ GalNAc1-  
17 4Gal $\beta$  containing glycans in these mucin producing cells. The nasal cavity in the infected mink thus  
18 shows features of carbohydrate expression comparable to what has been described in the respiratory  
19 system after *Pseudomonas aeruginosa* infection in humans.

20 *Conclusion:* It is suggested that the mink is suitable for studying *Pseudomonas aeruginosa*  
21 mediated rhino-sinusitis.

22 **Key words:** rhino-sinusitis, *Pseudomonas aeruginosa*, mink, mucin, sialic acid

## 1    **Introduction.**

2    We here evaluate the mink as a suitable animal model for experimental *P. aeruginosa* produced  
3    rhino sinusitis based on micromorphology and by use of antibodies and lectins directed against  
4    glycoconjugates.

5    *Pseudomonas aeruginosa* exists in nature in many forms and colonizes a number of different  
6    mucosal surfaces such as the respiratory, ocular, digestive, and reproductive mucosa. Epithelial  
7    cells from several tissues show a differential sensitivity to *P.aeruginosa* adherence [1].

8    The membranes of the airways in both man and mink are covered with a viscous colloid layer  
9    named mucus. The major components in mucus are mucins which are highly glycosylated proteins.  
10    *P aeruginosa* adhesins/lectins may play an important role for the colonization in immuno deprived  
11    patients since the bacterium adheres to mucins and other epithelial glycoconjugates [2-4]. The  
12    bacterium also causes colonization in most patients with cystic fibrosis (CF).

13    CF is caused by mutations in the transmembrane conductance regulator (CFTR) gene leading to  
14    decreased volume of airway epithelial lining fluid and letting go of mucus and thereby facilitating  
15    recurrent and chronic lung infections notably with *P. aeruginosa* growing as alginate containing  
16    biofilm [5-7]. Until now none of the transgenic cystic fibrosis mouse models that have been  
17    developed mimics the feature of human CF disease with chronic lung infection by mucoid strains of  
18    *P. aeruginosa*. [8-10]. The rabbit is often used as an animal model of sinusitis [11,12]. In this study  
19    we evaluate the mink as an alternative to study the pathogenesis of *p. aeruginosa* mediated sinusitis  
20    for several reasons. 1) The mucosal histology of both the upper and lower respiratory tracts in  
21    humans and mink are much alike. 2) *P. aeruginosa* is normally an organism of low invasiveness  
22    and low virulence in experimental laboratory animals and studies in the pathogenesis of its  
23    pathogenesis are hindered because of the inherent resistance of most species to disease of this

organism . However, it seems that the mink is the only animal species to develop fatal and contagious hemorrhagic pneumonia caused by *P. aeruginosa* [13-15]. 3) Mink are also susceptible to pulmonary infection with an experimental intra-tracheal or intra-nasal inoculation [16,17]. 4) We have previously shown that the lectins from *P. aeruginosa* bind to the submucosal glands and goblet cells in the mink airways [18].

Chronic rhino sinusitis with inflamed tissue and mucosal edema leads to decreased ciliary function and enhanced bacterial colonization. Consequently viscous mucus is present in most CF patients and the paranasal sinuses constitute an alternative colonization site [19]. The contaminating bacterium in the upper airways is predominantly *P. aeruginosa* as in the lungs [20].

Mucus forms a surface layer on the airways and serves as a first line of defense during airway infection. At least 16 mucin genes have been cloned in the lung and data suggest that some of them play a defensive role during pseudomonas infection [4,21].

Mucins are high-molecular weight, multifunctional glycoproteins that contain an elevated percentage of serine, threonine and proline in addition to a large quantity of complex O-linked oligosaccharides. The mucins are often classified as being membrane bound, or they are secreted non-gel-forming or secreted gel-forming mucins. In the airways they are produced by goblet cells in the surface epithelium and from submucosal glands. Mucins play a regulatory role in normal epithelial tissues. In the airways they protect and lubricate the respiratory tract [22]. They bind bacterial and viral pathogens as their anti-inflammatory role may be mediated through inhibition of toll-like receptor signaling [23]. In the present study we have explored the *in situ* binding to the nasal sections using antibodies directed against a number of mucins. Relevance for the mucins used for the study of infection (nomenclature for mucins: MUC in human; muc in mouse and Muc in other animal models (rat and chinchilla)).

1 **MUC1:** The mucin is a membrane-tethered mucin that interacts with *P. aeruginosa* through  
2 flagellin [24].

3 **MUC2:** Is highly elevated at the mRNA level in cystic fibrosis airways and following exposition *P.*  
4 *aeruginosa* [25].

5 **MUC3:** Seems not to be involved in pseudomonas infection. Expression of the mucin is therefore  
6 used as a negative control.

7 **MUC4:** The mucin was downregulated in mice infected with *Pseudomonas aeruginosa* [26]

8 **MUC5AC:** Is highly elevated at the mRNA level in cystic fibrosis airways and following  
9 exposition to *P. aeruginosa* [25]

10 **MUC5B:** The mucin is present in cystic fibrosis airway secretions [27].

11 **MUC16:** Is a membrane anchored mucin that that provides a barrier to bacterial adherence and  
12 invasion of epithelial cells [28].

13

14 When produced in excess, the mucus may contribute to the morbidity and mortality associated with  
15 respiratory diseases. Many mucins are highly sialylated and alteration of the sialic acid content are  
16 frequently associated with viscosity in mucins and impaired mucociliary transport leading to  
17 bacterial infection and inflammatory cell infiltration in the respiratory tract as in rhinosinusitis and  
18 bronchitis [29].

19 To study the impact of sialic acid epitopes in the normal and *P.aeruginos* inflamed tissue we used  
20 the following lectins and antibodies: *Maackia amurensis* lectin (MAA) which is specific for  
21 Sialyl $\alpha$ 2-3Gal [30], *Sambucus nigra* lectin (SNA) which is specific for the Sialyl $\alpha$ 2-6Gal linkage  
22 [31] . Anti-ganglioside GM1 that detects Gal $\beta$ 1-3GalNAc $\beta$ 1-4[Sialyl $\alpha$ 2-3]Gal $\beta$ 1-4Glc $\beta$ 1-ceramide  
23 and Anti-asialo-ganglioside GM1 that detects Gal $\beta$ 1-3GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc $\beta$ 1-ceramide.

The results will provide information on the use of mink as an experimental model for *P. aeruginosa* infection with inflammation and increased mucin production in the sinus-nasal cavity.



## 1    **Materials and methods.**

2    The mink were assigned to individual cages and with separate water supply. All animal experiments  
3    were conducted at the National Veterinary Institute, Technical University of Denmark in  
4    accordance with both institutional and national guidelines (Danish Animal Experiments  
5    Inspectorate, Permit Number: 2012-15-2934-00524). The experiments were approved by the Danish  
6    Animal Care and Ethics Committee, Denmark. The *P. aeruginosa* isolate used for infection was  
7    recovered from an outbreak of hemorrhagic pneumonia in mink on the Danish island Mors in 2008  
8    (Difco serotype O6, laboratory journal number at the National Veterinary Institute: 2008-52-937).  
9    This isolate was used because the O-antigen of B-band bacterial lipopolysaccharide contributes to  
10    initial damage and inflammatory responses in human lungs.

11    Four mink were infected intra-nasally with 0.5 ml *P. aeruginosa* with  $3.7 \times 10^3 - 10^7$  colony  
12    forming units (cfu)/ml 0.9% saline. The concentration of the bacterial solution was determined  
13    using the plate count method. Another four mink served as controls and received 0.9% saline intra-  
14    nasally.

15    Four days after infection the minks were euthanized. Swabs were taken from the nasal cavity and  
16    from the lung. For a more detailed procedure see Salomonsen et al. (13,14) The swabs were stroked  
17    on blood agar and grown at 37°C for 24 hours. The *P. aeruginosa* cultured from the nasal cavities  
18    had pulsed-field gel electrophoresis (PFGE) types indistinguishable from the isolate used for intra-  
19    nasal infection.

20    The mink were anesthetized with a ketamine/xylazin mixture injected intramuscularly and  
21    euthanized with an injection of T-61 (MDS-Animal Health, Boxmee, the Netherlands) in the liver.  
22    The nasal tissue samples were dissected immediately after the animals were euthanized.

The nasal tissue samples were prepared by cutting the nose in the dorsal plane right above the hard palate and transversally at the level of the eyes so that a large piece of the nasal cavity could be separated from the head. This nasal block was then fixed in 10% formalin for 1-2 days and demineralized in 14% EDTA with 1.5% w/v NaOH for 3 days, and embedded in paraffin.

The paraffin blocks were cut in 3 $\mu$ m sections and used for histochemical procedures

## **Histochemistry**

The following antibodies were used in this study:

Anti-MUC1 from Abcam (ab.1548) is a rabbit polyclonal antibody. The immunogen was a synthetic peptide with amino acid sequence from the cytoplasmic tail. Sections from human submandibular gland served as positive controls. Anti-MUC2 from Abcam (ab. 134119) is a rabbit monoclonal antibody. The immunogen was a synthetic peptide corresponding to a region with human MUC2. Sections from human colon served as positive controls.

Anti-MUC3 from biobyte (orb95411) is a rabbit polyclonal antibody. According to the manufacturer it reacts in tissues from human, mouse and rat. Anti-MUC4 from Santa Cruz (sc33654) is a mouse monoclonal IgG antibody raised against the transmembrane domain of rat mucin4. Anti-MUC5AC from MyBioSource (MBS2525387) is a rabbit polyclonal antibody. The immunogen was a synthetic peptide of human MUC5AC. Anti-MUC5B is a mouse monoclonal antibody from Abcam (ab77995). The immunogen was an un-glycosylated serine and threonine free, synthetic peptide from the N-terminal region of human MUC5B. Anti-MUC16 from Abcam (ab133419) is a rabbit polyclonal antibody. The immunogen was a synthetic peptide from internal sequence amino acids of human MUC16. Sections from human lung served as controls.

The following lectins and antibodies were used to demonstrate sialic acid residues. Biotinylated lectins from *Maackia amurensis* (MAA) and *Sambucus nigra* (SNA) (EY Laboratories; San Mateo, CA, USA). Sections from ferret lung tissue served as positive controls [32]. To study the binding of sialylated and non-sialylated glycosphingolipids we used antibodies against ganglioside GM1 and asialo-ganglioside GM1 [33]. Anti-GM1 from Calbiochem is rabbit polyclonal antibody and Anti-asialo-GM1 is a rabbit polyclonal antibody from LifeSpan.

Presence of phagocytic cells in the sections was demonstrated using an anti-myeloperoxidase antibody (Antibodies online Cat No: ABIN 1689910) that detects antimicrobial activity in inflamed tissue [34]. Presence of bacteria in the sections of the nasal tissue was detected with anti-Pseudomonas antibody from Abcam (catalog nr. ab 68538, according to the manufactures it was raised and validated with strain Boston 41501).

## 1 Results.

2 As in in the human organ, the mink nasal cavities are lined by a pseudostratified ciliated epithelium  
3 with goblet cells (Figs.1A and C). The lamina propria below the respiratory epithelium is richly  
4 vascular. The spaces between the turbinates (air channels) are normally empty but sparse cellular  
5 debris may be present. Both the human and mink submucosa contain exocrine glands but there  
6 fewer submucosal glands in the mink submucosa than in the human equivalent. An exception is the  
7 submucosa below the olfactory epithelium which contains numerous exocrine glands (Fig.1E).

8 The pseudostratified mucosal nasal epithelium was disintegrated and the loose connective tissue  
9 beneath the respiratory epithelium showed swelling and edema in the infected animals. A cellular  
10 accumulation was observed in the loose connective tissue in the turbinates and in the air channels  
11 from the *P. aeruginosa* inoculated mink (Figs.1B and D). The Alcian blue/PAS staining revealed  
12 that many cells in the debris between the turbinates were Alcian blue positive (Fig.1D). The  
13 connective tissue and the glands in the olfactory area of the nasal cavity was seemingly unaffected  
14 by the infection (Fig.1F) in contrast to what was observed in the respiratory part of the nasal cavity.

15 To decide if phagocytes had infiltrated the inflamed tissue some sections were incubated to show  
16 presence of myeloperoxidase containing cells. The turbinates in healthy mink displayed no sign of  
17 inflammation while the turbinate submucosa from the infected mink was loaded with  
18 myeloperoxidase positive cells (Figs.2A and B). Immuno-staining with an antibody against  
19 *Pseudomonas aeruginosa* served to verify bacterial accumulation in the inoculated animals. There  
20 was no immuno- reaction in sections from the control animals, while the air channels in the nasal  
21 cavities from the infected mink were loaded with strongly reacting debris (Figs.2C and D).

22 The expression of both MUC1 and MUC2 in the inoculated animals seems to be upregulated since  
23 there was a much stronger anti-MUC1 and anti-MUC2 staining in the respiratory epithelium from

the infected animals compared to the controls (Figs.3A-D). A sparse fine-granular anti-MUC1 reaction was present in the respiratory epithelium in the control mink while both the glycocalyx and the cell surfaces reacted strongly in the infected animals. MUC2 staining in the mucosal glycocalyx was noticed in both control and diseased animals. However, a strong i MUC2 reaction in the goblet cells was present only in the infected mink. Incubation with anti-MUC16 resulted in a weak and scattered staining in the surface epithelial cells from both groups of mink. . Incubation with anti MUC3 and anti-MUC4 showed no staining. A few goblet cells were stained in the control mink by anti-MUC5AC while numerous MUC5AC positive cells were observed in the infected animals (Fig 3E and F).

While the surface epithelium from both the healthy and infected mink displayed an intense fine-granular MUC5B reaction it was only the submucosa from the *P aeruginosa* infected mink that contained large MUC5B positive cells (Figs 4A and B). Potential changes in expression of sialic acid in pseudomonas infection were investigated by incubation with anti-GM1, anti-asialo-GM1, and the lectins MAA and SNA. Most of the respiratory epithelial cells showed a moderate staining intensity with anti-GM1 in both control and diseased animals. Incubation with asialo-GM1 resulted in strongly reacting with the nasal epithelial cells from the infected mink while the antibody did not stain the cells from the control mink (Figs.4C and D). The mucosal glycocalyx in both groups of animals reacted with the sialic acid detecting lectin MAA. However, the lectin staining revealed few MAA positive goblet cells in the sections from the healthy animals while there was numerous stained goblet cells in the sections from the inoculated animals (Fig E and F). Incubation with SNA resulted in staining of occasional goblet cells in both groups of animals.

## 1 Discussion.

2 The purpose of the study was to describe the pathological consequences of an experimental  
3 *P.aeruginosa* rhinosinitis in mink and thereby to evaluate the mink as an animal model for *P.*  
4 *aeruginosa* mediated infection. The same mink that were used in the present study have previously  
5 been examined for pathological changes in their lungs associated with *Pseudomonas aeruginosa*  
6 and it was concluded that the infected animals had developed typical hemorrhagic pneumonia [13,  
7 14].

8 To serve as a model for airway infections the mucosal histology in the respiratory tract from mink  
9 and humans should be much alike. In both species the nasal cavity, the paranasal sinuses and the  
10 tracheobronchial tree are lined by a pseudostratified columnar, ciliated epithelium with numerous  
11 goblet cells. The respiratory epithelium is supported by a richly vascular connective tissue  
12 containing exocrine glands.

13 The micromorphology of the mink and human airway systems is thus in many ways comparable.  
14 Lung and sino-nasal infections in humans with *P. aeruginosa* causes inflammation of the mucosal  
15 tissue [20,35,36] and we here show that the *P. aeruginosa* infected mink also expresses typical  
16 signs of inflammation and edema in the submucosa and disintegration of the nasal mucosa.

## 17 Inflammation markers.

18 Cells stained by the antibody directed against myeloperoxidase invaded the submucosa from the  
19 infected mink indicating the presence of neutrophils in the loose connective tissue [34]. Further, the  
20 air channels were stuffed with debris reacting with the antibody against *P. aeruginosa*. The *P*  
21 *aeruginosa* cultured from the nasal cavities had pulsed-field electrophoresis types indistinguishable  
22 from the isolate used for intranasal infection. We therefore conclude that the inoculation of the  
23 minks was successfully performed.

## 1 *Mucins.*

2 Changes of the carbohydrate sequences of respiratory could alter the mucus properties leading to  
3 ineffective mucociliary clearance and to bacterial colonization and infection [37]. The respiratory  
4 tract holds several mucin types. We have focused on the expression of some mucins that all have  
5 been detected in the human airways.

## 6 *MUC1*

7 MUC1 is a transmembrane mucin which is expressed on the apical surface of many epithelial cells  
8 as well as hematopoietic cells. The mucin is primarily involved in the protection of epithelial  
9 surfaces and contributes to the mucosal barrier to infection. MUC1 seems to play an important role  
10 in *P. aeruginosa* infections since the mucin suppresses inflammatory responses induced by the  
11 bacterium. Measurements of lung tissue lysates have shown that MUC1 levels in wild type mice is  
12 initially low, but gradually increases after *P. aeruginosa* inhalation [38] and the mucin may prevent  
13 lung injury in mice models of repetitive *P. aeruginosa* infection [39].

14 The mucin contributes to the mucosal barrier to infection and the mucin suppresses inflammatory  
15 responses induced by *P. aeruginosa*. Lillehoj *et al.* [40,41] have showed that MUC1 mucins on the  
16 epithelial cell surface are adhesion sites for *P. aeruginosa* flagellin. We observed that only a weak  
17 granular reaction was present in the respiratory epithelium in the control mink after incubation with  
18 anti-MUC1 while both the glycocalyx on the cell surface and the cell membranes in the respiratory  
19 epithelial cells reacted strongly in the infected animals.

## 20 *MUC2*

21 The surface epithelium in the intestine is coated with a thick protective MUC2 mucin while this gel-  
22 forming mucin is absent or very scarcely present in the mucous gel of normal airways [42] *P.*

*aeruginosa* infection leads to upregulation of the mucin gene MUC2 in both humans and animals [43,44]. The upregulation of MUC2 during airway inflammation induced by bacterial infection could be important to protect the tissue from inflammatory cells

We here show that the nasal surface epithelium in both the healthy and the infected mink was coated with a glycocalyx that stained strongly after incubation with anti-MUC2. A cytoplasmic reaction was however only noticed in the epithelial cells infected with *P. aeruginosa*. Mucin2 is a glycoprotein that is secreted predominantly from the goblet cells and it seems that intracellular staining of anti-MUC2 in the epithelial cells suggests that *P. aeruginosa* can stimulate secretion of this mucin in the airways.

*MUC3, MUC5AC, MUC5B, MUC16.*

MUC3 is a transmembrane glycoprotein that is expressed in normal colon and in very low levels in the respiratory tissues [45]. In contrast to the strong activity we noticed with the transmembrane mucin MUC1 our results show no immunostaining with anti-MUC3 in *P. aeruginosa* infected mink. Both mucin 2 and mucin 5AC are produced in goblet cells. We noted an increased immunostaining in the goblet cells with anti-MUC2 and anti-MUC5AC after *P. aeruginosa* infection but the glycocalyx reacted only with anti-MUC2. Chorley et al [46] likewise noticed a differential MUC2 and MUC5AC secretion by tracheal cells. MUC5B is required for airway defense and Sepper et al [47] found that expression of the mucin in lung alveolar macrophages was increased in long-term smokers and in mice infected by multiple bacteria there was an increased secretion of Muc5B and macrophage activity in the airways [48]. We demonstrated large Muc5B positive macrophage-like cells in the nasal submucosa. Also MUC16 are attributed to airway goblet cells [40]. Muc16 is a cell surface mucin that provides a barrier to bacterial adherence but only a few MUC16 positive cells were observed in the two animal groups.



Increased production of airway mucins seems to be prerequisite in establishing a bacterial biofilm in the airways. The virulence of *P. aeruginosa* is partly dependent by its ability to form a biofilm and this bacterial biofilm contributes to the pathogenesis of chronic rhino-sinusitis. We show increased staining of antibodies directed against mucin1, mucin2 and mucin5AC and mucin 5B in the nasal turbinates from mink infected with *P. aeruginosa*.

### ***Sialic acids.***

Sialic acids are nine-carbon sugar residues present on glycosphingolipids, N-glycans and O-glycans. *N*-Acetylneuraminic acid (NeuAc) is the exclusive sialic acid in humans and thus often referred to simply as sialic acid. The sugar occupies the terminal position within glycan molecules on the surface of vertebrate cells and serves as receptors for many pathogenic bacteria and viruses. Inflammation may modify the appearance of glycoproteins [41] and carbohydrates containing sialic acid change level of expression in the respiratory tract during infection . Gallego and Hulen [1] have shown that variable amount of sialic acid in the epithelial cell surface glycoconjugates causes differential sensitivity to *P. aeruginosa* adherence.

It has long been established that mucins and sialic acid act as receptors for *P. aeruginosa* in the respiratory tract [49,50]. Mucins are rich in sialic residues and sialylation may be altered in the mucin in CF patients suffering from *P. aeruginosa* infection. In the present study we have used lectins and glycosphingolipids as markers to detect presence sialic acid epitopes in the mink nasal tissue. Some studies have shown a shift from a  $\alpha$ 2-6 sialic acid linkage to a  $\alpha$ 2-3 sialic acid linkage [51]. MAA is a lectin that binds with high affinity to oligosaccharides containing terminal sialic acid linked 2-3 to penultimate galactose residues. We here show that in the infected nasal epithelium it is the goblet cells that are upregulated with  $\alpha$ 2-3 sialic acid containing glycans.

1 Incubation with SNA implies that there was no upregulation of  $\alpha$ 2-6 sialic acids in mink nasal  
2 tissues.

3 Sialic acid is not the only carbohydrate-receptor that is vital for binding *P. aeruginosa*. The  
4 glycosphingolipid GM1 contains one sialic acid residue bound to galactose. Incubation with this  
5 ganglioside showed no differences in the sparse immuno-staining between sections from infected  
6 and non-infected animals. When the sialic acid residue is removed from the ganglioside GM1 as in  
7 asialo-ganglioside GM1 then antibody staining showed no reaction in the nasal tissue from the  
8 control mink while there was a strong asialo-GM1 expression in the infected tissue. This result is in  
9 concordance with Bentzmann et al [52]) who observed that the pili of *P. aeruginosa* bind  
10 specifically to the carbohydrate sequence  $\beta$ GalNAc1-4Gal $\beta$ , a disaccharide sequence present in  
11 asialo-GM1.

12 A marked association exists between upper and lower airway cultures in patients with CF since the  
13 paranasal sinuses often are colonized with matching CF lung bacteria [53] and treatment of the  
14 infection in both the lung and paranasal sinuses is thus important [54].

15

## 16 **Conclusions.**

17 Since the nasal cavity from mink with acute *P. aeruginosa* infection displays carbohydrate  
18 expression comparable to what is described as a chronic biofilm-based infection in the respiratory  
19 system we suggest that the mink can be used to study *P. aeruginosa* mediated rhino-sinusitis. The  
20 genome of the mink seems not yet to have been sequenced which is a serious drawback when it  
21 comes to design molecular reagents for the use of experiments with this animal.

## 22 **Authors contributions**

ASH, NH, CMS conceived and designed the experiments. CMS performed the experiments. SK was responsible for the histochemistry and wrote the manuscript

The authors declare that they have no conflict of interest.

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## 1 **Illustrations.**

2 **Fig.1.** Sections of healthy control mink (Figs. A, C and E) and mink that were inoculated with  
3 *Pseudomonas aeruginosa* (Figs B, D and F) stained with HE-PAS-Alcian blue.

4 Control animals: The nasal cavity is covered with continuous respiratory epithelium. Two turbinates  
5 are shown in Figs.A and C. The green arrow in Fig. A marks the osseous part of a turbinate. In  
6 Fig.C the goblet cells are marked by a green arrow. The goblet cells are present in the columnar  
7 epithelial cells and stained by Alcian Blue. The cytoplasm in the columnar cells and the loose  
8 connective tissue underneath the surface epithelium (the submucosa, marked S) is stained pink by  
9 eosin. The olfactory mucosa is composed of a pseudostratified epithelium that contains different  
10 cell types (Fig.E). The olfactory cilia are non-motile and are marked by an arrow. They are strongly  
11 stained by Alcian Blue. Beneath the epithelium (O) there is a loose vascular connective tissue with  
12 afferent nerves and many Alcian Blue positive exocrine glands (Bowmans glands; G).

13 Infected animals: Following the intra-nasal infection the connective tissue in the turbinates became  
14 swollen with edema and cellular infiltration and the surface epithelium was disintegrated (Fig.B).  
15 The green arrow in Fig.B marks the osseous part of a turbinate.

16 While the air channels contained no or few cells in the healthy mink they were filled with cellular  
17 debris (DE) in the infected animals. Many of the debris cells are stained by Alcian Blue (Fig.D).

18 In the olfactory region the morphology of the surface epithelium and the supporting connective  
19 tissue and the glands seem to be unaffected by bacterial inoculation (Fig. F).

20 **Fig. 2.** Immunohistochemical staining for myeloperoxidase in a turbinate from a normal (Fig.A) and  
21 from a diseased (Fig.B) animal. The contours of the turbinates are outlined with white line The  
22 submucosa in Fig.B contains many positive cells. Figs.C and D show the staining in the nasal

cavities after incubation with the anti-pseudomonas aeruginosa antibody. The cellular debris (DE) in the air channels from the infected mink reacts strongly. The contours of the turbinates are outlined with white lines.

**Fig. 3.** Figs.A-D illustrate the changes that takes place in mucin expression in the nasal turbinates after infection with *Pseudomonas aeruginosa*. The sections were stained with antibodies against MUC1 (Figs.A and B) and MUC2 (Figs.C and D). Figs. A and C show tissue from a healthy animal and Figs.B and D show tissue from an infected animal. The respiratory surface epithelium (EP) from the healthy animal was nearly non-reacting after incubation with anti-MUC1 while the epithelium from the infected mink was strongly stained. The antibody directed against MUC2 reacted with the glycocalyx (marked by an arrow) from both the control (Fig.C) and the inoculated (Fig.D) animal while only the underlying respiratory epithelium from the sick animal showed anti-MUC2 staining. Figs.E and F illustrate reaction for MUC5AC. Only few goblet cells are stained positive in the respiratory epithelium from the control mink (Fig.E) while there are numerous MUC5AC positive goblet cells in the epithelium from the inoculated animal (Fig.F).

**Fig.4.** Reaction pattern in the turbinates for MUC5B is shown in figs. A and B. A small-granular reaction is seen in the surface epithelium from both the infected and the healthy mink. The subepithelial tissue contains from the inoculated animals contains large MUC5B positive cells marked by arrowhead. Reaction pattern in the turbinates for asialo GM1 is depicted in Figs.C and D. There is virtually no reaction in the turbinates from the healthy mink (Fig.C) while almost all the epithelial cells react in the cells from the infected animal (Fig. D). Thickness of the epithelium is indicated by arrows. Figs.E and F show MAA reaction in the turbinates. The goblet cells (marked by green arrows) in the respiratory epithelium from the healthy mink were almost blank after incubation with the lectin (Fig E) while they were strongly stained in the infected animals (Fig.F).

